Research Article

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Evidence Against Combined Effects of Stress and Brain Stimulation on Working Memory

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Abstract: The effect of stress on working memory has been traced back to a modulation of the prefrontal cortex (PFC). We investigated the effects of neuromodulation of the left dorsolateral prefrontal cortex (lDLPFC) after exposure to psychosocial stress through the Socially Evaluated Cold Pressure Test (SECPT). The hypothesis was that neuromodulation interacts with the stress intervention, to either boost performance even under stressed conditions or compensate negative stress effects. Fifty-nine participants were randomly divided into two groups. One group received active, anodal, offline transcranial direct current stimulation (tDCS) over the lDLPFC while the other group received sham stimulation. Participants performed a lexical n-back task, before and after the SECPT and tDCS intervention. The first n-back task was used as a baseline measurement and the second n-back task was performed during recovery from stress when cortisol levels are at their peak, but still under the influence of tDCS aftereffects. Additionally, after the psychosocial stress phase participants were post-hoc divided into cortisol responders and non-responders. Results showed that generally stress increased lexical n-back task performance as indicated by faster correct reaction times and higher accuracy but that this was not modulated by tDCS. Crucially, using Bayes analysis we obtained evidence against the influence of anodal tDCS on stressed individual's working memory performance.

Keywords: transcranial direct current stimulation, stress, cortisol, working memory, nback

Introduction

Unexpected stimuli and situations that can threaten homeostasis can elicit a physiological and psychological stress response. This stress response involves a surge in arousal, enhanced vigilance and a shift in cognitive processing (De Kloet, Joëls, & Holsboer, 2005). In detail, stress effects on cognition are dependent on the stress intensity and are related via an inverted U-shaped function. Put simply, cognitive performance is best if a moderate stress response occurs, whereas drastically higher stress intensities hamper cognitive functioning (e.g. Lupien, & McEwen, 1997; McEwen, 2004; Arnsten 2009; Salehi, Cordero, & Sandi, 2010; Sapolsky, 2015). Depending on the situation – for example during medical procedures – a drop in performance due to psychosocial stress might be detrimental. One way to potentially enhance cognitive performance, in general, is transcranial direct current stimulation (tDCS) (e.g. Coffman, Clark, & Parasuraman, 2014; Friehs & Frings, 2018; Friehs & Frings, 2019; Frings et al., 2018; Friehs et al., 2019; Jacobson, Koslowsky, & Lavidor, 2012; Loftus et al., 2015; Mancuso, Ilieva, Hamilton, & Farah, 2016; Summers, Kang, & Cauraugh, 2015). In the present study we explore the interaction effects of stress and tDCS with regards to working memory (WM). Specifically, we hypothesized that tDCS either compensates the negative stress effects on cognition

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or boosts positive stress effects on WM. Our results demonstrate that stress can positively affect WM and that synchronized tDCS has no effect on stressed individuals with a cortisol response.

Working Memory and its Neural Correlates

A widely used measure of WM functioning is the n-back task, in which subjects are presented with a continuous stream of stimuli and are instructed to press a key when they detect a repetition at a specified delay. Depending on the desired task difficulty, the delay can be either increased or decreased. Conditional on the task difficulty and the stimulus material (e.g. shapes, locations, numbers, letters), the n-back task evokes widespread activation within the prefrontal cortex (for meta-analysis see Owen, McMillan, Laird, & Bullmore, 2005; Rottschy et al., 2012). Drawing on those results, the left dorsolateral prefrontal cortex (lDLPFC) has been consistently implicated in lexical and verbal WM processes (e.g. Barch, Sheline, Csernansky, & Snyder, 2003; D'Esposito et al., 1995; D'Esposito, Postle, & Rypma, 2000).

tDCS Effects on Working Memory

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation tool that utilizes a constant electric current flow between an anode and a cathode (Miranda, Lomarev, & Hallett, 2006). tDCS can be applied during the to-be-modulated task (*online* stimulation) or right before it (*offline* stimulation). Depending on the timing of stimulation, different mechanisms of action are engaged that either revolve around modulation of membrane potentials (in the case of online tDCS) or modification of synaptic and neurotransmitter activity (in the case of offline tDCS). Furthermore, depending on whether the anode or the cathode is positioned over a certain brain area, the stimulation might be either excitatory or inhibitory (Miniussi, Harris, & Ruzzoli, 2013; Stagg et al., 2009; Stagg & Nitsche, 2011).

WM has been a focus of tDCS research, with a multitude of meta-analysis and reviews written on the topic already (e.g. Brunoni & Vanderhasselt, 2014; Jantz, Katz, & Reuter-Lorenz, 2016; Mancuso, Ilieva, Hamilton, & Farah, 2016 but see also Horvath, Forte & Carter, 2015a,b). While newer reviews and meta-analysis show promising effects of tDCS on WM, there are also many studies that fail to replicate such findings. And even though some issues are still up for debate, such as the influence of the timing of the stimulation (Friehs & Frings, 2019b), it seems that anodal tDCS over the PFC can impact WM performance. Specifically for the n-back task, studies have reported a positive effect of single-session anodal tDCS over the DLPFC on WM (e.g. Fregni, Boggio, Nitsche, & Bermpohl, 2005; Hoy et al., 2013; Ohn et al., 2008).

Stress and its Effects on Cognition

In response to acute stress, activation of the sympathetic nervous system is quickly increased, which is mediated by the catecholamines adrenaline and noradrenaline. The slower, metabolic stress response involves a system termed the HPA-Axis (Hypothalamus-Pituitary-Adrenal Axis) (De Kloet et al., 2005), which cumulates in a secretion of cortisol into the bloodstream (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009; Herman, Ostrander, Mueller, & Figueiredo, 2005). Within the human brain, several different areas are involved in the interpretation of, and reaction to, a stressful situation. Chief among those are the prefrontal cortex (PFC), the amygdala and hippocampus (McEwen, 2004). In interaction with the hippocampus and amygdala the DLPFC plays a crucial role in directing attention to the most relevant stimuli and reducing attention to irrelevant, distracting stimuli. The PFC is especially sensitive to the lingering effects of stress due to the high density of glucocorticoid receptors (Arnsten, 2009, 2015), which is in line with reports that show a widespread deactivation of the executive control network directly following the stressor (Hermans, Henckens, Joëls, & Fernández, 2014)

It has been put forward that stress and cognition are connected through an inverted U-shaped function. This means that depending on the conditions and cortisol concentration, cognitive functioning might be enhanced or impaired (Andreano & Cahill, 2006; Jelici, Geraerts, Merckelbach, & Guerrieri, 2004; Lupien, & McEwen, 1997; McEwen, 2004; Vedhara, Hyde, Gilchrist, Tytherleigh, & Plummer, 2000). This might also

partially explain contradicting results with regards to the relation between stress and WM functioning: while some studies report a decrease in WM functioning after stress (Qin, Hermans, van Marle, Luo, & Fernández, 2009; Robinson, Sünram-Lea, Leach, & Owen-Lynch, 2008; Schoofs, Preuß, & Wolf, 2008), some report null effects (Hesse, Schroeder, Scheeff, Klingberg, & Plewnia, 2017) or even an improvement (Lupien, Gillin, & Hauger, 1999; Weerda, Muehlhan, Wolf, & Thiel, 2010).

The Present Study

The overall goal of the present study is to investigate the interaction of tDCS and stress effects on working memory performance. Both stress and tDCS impact WM performance via the PFC. Yet, while the effects of anodal tDCS on WM are quite coherent in the literature, the effects of stress are less clear. Thus, we expected a potential reversing of negative stress effects on WM due to tDCS or a further boosting due to tDCS. To this end, we implemented two tDCS conditions, contrasting anodal and sham tDCS over the left DLPFC in a repeated measures design, while both tDCS groups underwent the socially-evaluated cold pressor test (SECPT) in order to induce a stress reaction (for an overview of the study design see **Figure 1A**). Stress was characterized by cortisol response and thus only cortisol responders were considered for working memory performance analysis. Comparison between the stimulation groups allows us to test whether or not tDCS might be able to enhance WM performance under stressed conditions (i.e. under cortisol influence).

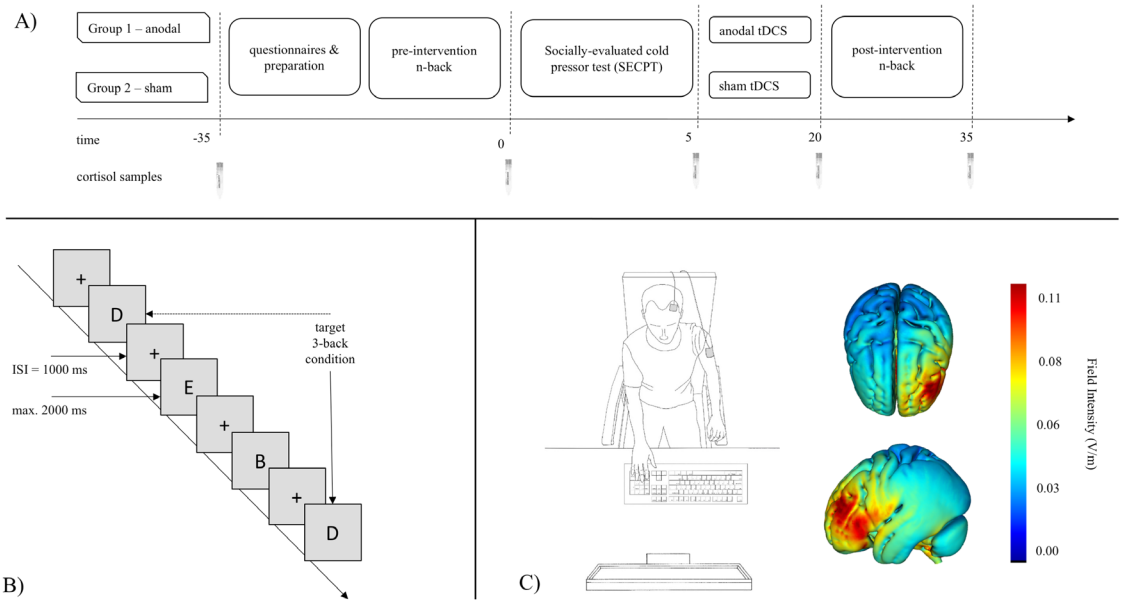

Figure 1. Depiction of elements of the study design. **A)** Schematic representation of the study procedure and design. **B)** Example of a trial sequence and stimulus material. **C)** tDCS application: the anode (9 cm²) was always positioned over the left DLPFC while the cathode (35 cm²) was placed over the left deltoid. Direct current flow during anodal stimulation using the HD-Explore software (version 3.0, Soterix Medical Inc, New York).

Experiment

Method

Sample. Sixty healthy adults were recruited for the study, but one participant voluntarily dropped out during the stress-test. This resulted in a final sample of fifty-nine, right-handed subjects (forty female, mean age

= 23.53 ± 4.31). All participants had normal or corrected to normal vision. No subjects had any prior history of neurological, cardiovascular or psychiatric illness. Furthermore, subjects were excluded if they recently consumed illegal drugs or alcohol the previous night. The local ethics committee of the University of Trier approved the study. All participants provided written informed consent.

tDCS. A 4-channel-DC-stimulator by NeuroConn, Ilmenau provided a constant direct current. In the present study, two different stimulation conditions were implemented: an active stimulation condition utilizing anodal offline tDCS and a sham stimulation condition. In the anodal as well as the sham condition one electrode of 9 cm² (3 x 3 cm) was positioned over the left DLPFC (F3 Position according to the extended 10-20 electrode reference system (Chatrian, Lettich, & Nelson, 1988), while the 35 cm² (5 x 7 cm) reference electrode was applied over the left deltoid muscle (**Figure 1C**). In the anodal stimulation condition a constant current of 0.5 mA was applied for 19 min, with additional 30-second ramp up and ramp down periods at the start and end of the simulation. This resulted in a current density of 0.056 mA/cm² and 0.014 mA/cm² respectively. In the sham condition, a ramp up/ramp down phase of 30 sec each was included at the start and right at the end of the supposed stimulation.

Saliva sampling and cortisol analysis. Subject collected saliva themselves using a commercially available sampling device (Salivette; Sarstadt ®, Nümbrecht-Rommelsdorf, Germany). Cortisol was sampled five times throughout the experiment: (1) upon entering the lab, thirty-five minutes before the SECPT, (2) after the first n-back session right before the SECPT, (3) five minutes after onset of the stressor or immediately following the SECPT, (4) twenty minutes after the onset of the stressor, following directly after the tDCS and (5) thirty-five minutes after the onset of the stressor, after the second n-back session. Thus, there are two baseline measures before the SECPT, one directly after the stressor and two samples that should mirror the peak-cortisol response. Samples were stored at -20°C until analysis. For the analyses of free cortisol, samples were thawed and centrifuged at 2000 g for 10 minutes to obtain 0.5 – 1.0 ml clear saliva with low viscosity. The fraction of free cortisol in saliva (i.e. salivary cortisol) was determined using a time-resolved immunoassay with flurometric detection (Dressendörfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992).

Autonomic stress response. Heart rate was assessed using the ECG ANS-Recorder (Neurocor®, produced by TELMED, Germany) which was mounted to the participants chest. ECG signals were analysed using the Neurocor ANS-Explorer software (version 3.4.3). Heart rate was monitored continuously and four intervals (i.e. between each of the five cortisol sampling points) were defined for later analysis. Larger time intervals were necessary to analyze Heart-Rate-Variability (HRV). Specifically, normalized low- and high-frequency power bands (nuLF and nuHF) were extracted, which could also be used to calculate the sympatho-vagal balance (LF/HF).

SECPT. The SECPT was modelled after Schwabe, Haddad, & Schachinger (2008). The participants were informed that a video recording would be made for scientific evaluation of facial expressions. Subjects were asked by a second, stern-looking experimental, with a white lab coat, to immerse both feet, including their ankles, into rolling ice water (0 – 3°C). Subjects were instructed by the experimenter responsible for the stress task to look into the camera, refrain from talking and keep their feet immersed in water. The experimenter observed the participants the entire time and took notes. If participants kept their feet immersed for 3 minutes, they were instructed to remove them.

n-back task. Participants were seated in front of a 19-inch color monitor with a viewing distance of 65 cm in a normally lit room. Participants responded only using their right hand by pressing one of two marked keys on a keyboard in front of the monitor; one key for identification of the target and the other for rejection of a target. The n-back task was implemented using the E-Prime Software, Version 2.0. Before the first 3-back measurement participants were given the opportunity to familiarize themselves with the n-back task. To this end a 0-, 1-, 2- and 3-back condition with 20 trials each was implemented. The first as well as the second 3-back measurement consisted of 288 trials (25% of which were target trials) divided into 4 blocks

of 72 trials. N-back Stimuli consisted of the first eight letters in the English alphabet. Before a stimulus was presented a fixation cross appeared on screen for 1000 msec, which was afterwards replaced by the stimulus. The stimulus was presented until reaction or a maximum of 2000 msec (**Figure 1B**).

Procedure. All experimental sessions started between 14:00 and 18:00h to control for the diurnal cycle of cortisol (Weitzman et al., 1971; De Kloet, Oitzl, & Joëls, 1999; De Kloet, Joëls, & Holsboer, 2005; Buckley & Schatzberg, 2005). After arrival at the laboratory, the participant was randomly assigned to one of two conditions: (1) anodal offline tDCS of the left DLPFC in combination with a SECPT session or (2) sham tDCS of the left DLPFC in combination with a SECPT session. Each experimental session followed a standardized procedure (1) fill out a questionnaire concerning the exclusion criteria and demographic data, (2) mounting of electrodes for ECG and tDCS (3) pre-tDCS n-back task (4) SECPT and following a subjective stress rating (5) 15min of either anodal or sham tDCS[1] (6) post-tDCS n-back task and lastly (7) side effects questionnaire and hair cleaning. The whole experiment took around 120min from entering to exiting the lab. Importantly, the experimenter administering the SECPT was blind to the tDCS condition.

Design. The study was based on a 2 (session: pre-tDCS vs. post-tDCS) x 2 (trial type: targets vs. distractors) x 2 (tDCS condition: anodal vs. sham) design with tDCS condition being varied between participants. Furthermore, participants were post-hoc classified as cortisol-responders or non-responders depending on their cortisol response to the SECPT; the quasi-experimental two-level factor *cortisol response* (responder vs. non-responder) was then added to the analysis. For analysis of WM performance, only cortisol responders were considered.

Results

Physiological stress response indicators such as cortisol response and heart-rate are depicted in **Table 1** and **2** respectively. RT means and accuracy rates are depicted in **Table 3**. For a detailed between-groups comparison of cortisol response see **Table A1** and **A2** in the appendix. **Table A3** contains the BFs comparing both stimulation groups with regards to physiological measures.

Table 1. Mean cortisol concentrations, standard deviations are depicted in brackets.

		-35 min	0 min	+5 min	+20 min	+35 min
Overall		4.69 (3.19)	4.34 (2.34)	4.10 (2.17)	8.75 (5.53)	7.91 (6.56)
tDCS – condition						
	Anodal	4.11 (2.77)	3.86 (1.72)	3.72 (1.99)	7.49 (3.28)	6.60 (3.34)
	Sham	5.26 (3.51)	4.79 (2.77)	4.47 (2.31)	9.96 (6.90)	9.17 (8.48)
Responder vs. non – responder						
	Responder	4.32 (2.94)	4.22 (2.34)	4.03 (2.27)	9.71 (5.74)	8.78 (7.06)
	Non – responder	6.16 (3.84)	4.77 (1.95)	4.39 (1.78)	4.99 (2.03)	4.49 (1.62)

1 Since there is evidence that suggest cognitive effort during stimulation can affect stimulation after-effects, participants were handed several easy-to-read, non-emotional or cognitively taxing magazines (e.g. nature documentary magazines) in order to stop participant from engaging in mind-wandering behaviour and provide a standardization of cognitive effort across participants (Horvath, Carter, & Forte, 2014).

Table 2. Mean heart rate in beats-per-minute (BPM) and HRV parameters at certain times throughout the experiment split by tDCS condition. Standard deviations are depicted in brackets.

	anodal tDCS				**sham tDCS**			
	first n-back	**during SECPT**	**during tDCS**	**second n-back**	**first n-back**	**during SECPT**	**during tDCS**	**second n-back**
BPM	79.35 (15.28)	86.18 (17.21)	69.71 (8.92)	72.68 (8.92)	78.01 (9.99)	87.45 (14.04)	71.66 (9.07)	75.45 (10.28)
nuLF	68.21 (14.29)	62.73 (15.86)	56.41 (15.80)	61.66 (15.45)	72.97 (11.10)	70.81 (16.90)	65.68 (16.69)	72.03 (10.69)
nuHF	31.79 (14.29)	37.27 (15.86)	43.59 (15.80)	38.34 (15.45)	27.03 (11.10)	29.19 (16.90)	34.33 (16.69)	27.97 (10.69)
LF/HF	2.75 (1.51)	2.24 (1.55)	1.70 (1.31)	2.18 (1.60)	3.35 (1.77)	3.52 (2.21)	2.75 (1.98)	3.25 (2.19)

Table 3. This table shows the change – specifically the improvement – in corrects RTs in msec and accuracy rates in percent from session 1 to session 2. The table is split into part **A)** which displays the change from session 1 to 2 with regards to distractor stimuli and part **B)** which represents the change with respect to target stimuli.

A) **distractors**				
	Δ accuracy		Δ correct RTs	
	responder	non - responder	responder	non - responder
anodal tDCS	.01 (.05)	-.01 (.09)	120.70 (135.44)	77.57 (79.70)
sham tDCS	.02 (.04)	.01 (.04)	127.43 (99.85)	70.88 (90.21)
B) **targets**				
	Δ accuracy		Δ correct RTs	
	responder	non - responder	responder	non - responder
anodal tDCS	.06 (.14)	.10 (.16)	116.26 (130.56)	16.89 (73.17)
sham tDCS	.05 (.09)	-.03 (.16)	117.20 (127.63)	146.82 (95.75)

Analyses focus on the impact of both stress (as determined by cortisol response) and tDCS on WM performance. Overall results showed that stress increased lexical n-back task performance as indicated by faster correct reaction times and higher accuracy. Furthermore, we obtained evidence against the modulation of stress effects via anodal tDCS.

Cortisol. Firstly, it was established that a significant cortisol response was elicited by the SECPT and that this physiological reaction was independent of the applied tDCS. Secondly, results show that responder and non-responders significantly differ in their cortisol response. And thirdly, a combined analysis shows that sampling time, tDCS condition and the strength of the cortisol-response do not interact. Furthermore, the results also show that the second n-back session was performed during the peak cortisol response.

Firstly, cortisol samples were submitted to a 5 (sampling time: -35 vs. 0 vs. +5 vs. +20 vs. +35) x 2 (tDCS condition: anodal vs. sham) mixed-measures MANOVA. The main effect of sampling time was significant ($F(1, 54) = 17.01$, $p < .001$, $\eta_p^2 = .56$), while the main effect of tDCS condition was not ($F(1, 57) = 3.89$, $p = .053$). Importantly however, the interaction between sampling time and tDCS did not reach significance ($F(1, 54) < 1$, $p = .58$). To further explore this analysis, the minimum baseline cortisol level and the maximal cortisol response after the SECPT were submitted to a 2 (cortisol level: baseline vs. peak) x 2 (tDCS-condition: anodal vs. sham) ANOVA. As indicated by the main effect of cortisol level, the rise in salivary cortisol from the minimal baseline level to the maximum post-stress level was statistically significant ($F(1, 57) = 40.69$, $p < .001$, $\eta_p^2 = .42$). The main effect of tDCS-condition reached significance ($F(1, 57) = 4.03$, $p < .05$, $\eta_p^2 = .07$), indicating an overall slightly higher cortisol concentration in the sham condition. The interaction between the tDCS-condition and the cortisol response was non-significant ($F(1, 57) = 1.89$, $p = .18$), indicating that the tDCS did not influence the cortisol response. Cortisol means can be seen in **Table 1**.

Secondly, following the recommendations by Miller, Plessow, Kirschbaum, & Stalder (2013) participants

were split into responder and non-responder depending on whether or not an increase of 1.5nmol/l in cortisol concentration could be observed. This resulted in a group of twelve non-responder and forty-seven cortisol responder. A 5 (sampling time) x 2 (cortisol response: responder vs. non-responder) MANOVA was conducted resulting in a significant main effect of sampling time ($F(1, 54) = 7.89$, $p < .001$, $\eta_p^2 = .37$) and an interaction between sampling time and cortisol response ($F(1, 54) = 5.17$, $p < .001$, $\eta_p^2 = .28$). The main effect of cortisol response was not significant ($F(1, 57) = 1.54$, $p = .22$). To better understand this interaction, a 2 (cortisol response: responder vs. non-responder) x 2 (cortisol level: baseline vs. peak) ANOVA was conducted. The cortisol difference between responder and non-responder can be inspected in **Figure 2** and **Table 1**.

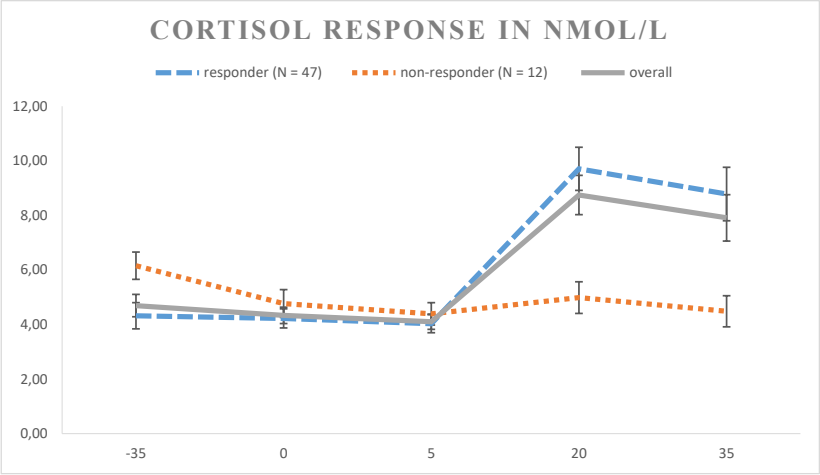

Figure 2. Salivary cortisol in nanomoles per liter (M ± SEM) at several time points across the experiment. Cortisol responses significantly increased after the SECPT overall. The dotted lines represent cortisol responder and non-responder respectively.

Thirdly, a 2 (tDCS condition: anodal vs. sham) x 2 (cortisol response: responder vs. non-responder) x 2 (cortisol level: baseline vs. peak) MANOVA was carried out. Results importantly only show a significant main effect of cortisol level ($F(1, 55) = 13.29$, $p < .001$, $\eta_p^2 = .20$) and a significant interaction between cortisol level and cortisol response ($F(1, 55) = 9.68$, $p < .01$, $\eta_p^2 = .15$). The main effects of cortisol response ($F(1, 54) = 3.14$, $p = .08$) and tDCS condition ($F(1, 55) = 1.29$, $p = .26$) were not significant. The interaction between cortisol level and tDCS condition, the interaction between cortisol response and tDCS condition, as well as the three-way interaction, all yielded statistically insignificant results (F's < 1)

Thus, taken together it can be assumed that the SECPT deployed in the present study did result in a significant stress response, which was not dependent on or altered by tDCS. Furthermore, the analysis of cortisol response allowed for a clear classification of responders and non-responders based on their cortisol response.

Heart Rate. The following analyses show that the participants' heart rate was significantly elevated during the stress test. Furthermore, analyses of frequency bands revealed that after the SECPT (i.e. during tDCS), LF power decreased while HF power increased indicating a higher parasympathetic activation, which is in concert with the lower heart-rate following the SECPT.

The heart rate, as measured by beats-per-minutes (BPM), was submitted to a 4 (sampling time: during first n-back vs. during SECPT vs. during tDCS vs. during second n-back) x 2 (tDCS condition: anodal vs sham) x 2 (cortisol response: responder vs. non-responder) MANOVA. The only effect that reached statistical significance was the main effect of sampling time ($F(1, 51) = 21.53$, $p < .001$, $\eta_p^2 = .56$). Post-hoc planned simple-contrasts revealed that the heart-rate during the SECPT (see **Table 2**) was significantly elevated; BPM during SECPT vs. during first n-back ($F(1, 53) = 26.89$, $p < .001$, $\eta_p^2 = .34$), during tDCS ($F(1, 53) = 60.66$, $p < .001$, $\eta_p^2 = .54$) and during the second n-back ($F(1, 53) = 46.96$, $p < .001$, $\eta_p^2 = .47$). The main effects of tDCS

condition ($F(1, 53) = 1.48$, $p = .23$) and the main effect of cortisol response $F(1, 53) = 0.09$, $p = .76$), as well as their interaction ($F(1, 53) = 1.89$, $p = .18$) were not significant. The interactions between sampling time and cortisol response ($F(1, 51) = 1.49$, $p = .23$), between sampling time and tDCS condition ($F(1, 51) = 0.26$, $p = .85$) and between all three variables ($F(1, 51) = .34$, $p = .80$) were non-significant.

Heart-Rate-Variability (HRV) analyses were performed on three different, convergent measures: the normalized low-frequency band (nuLF) and the normalized high-frequency (nuHF) of the HRV, as well as the power-ratio of LF and HF bands (LF/HF). All variables were separately submitted to a 4 (sampling time: during first n-back vs. during SECPT vs. during tDCS vs. during second n-back) x 2 (tDCS condition: anodal vs sham) x 2 (cortisol response: responder vs. non-responder) MANOVA. The pattern of results stayed consistent across measures.

Analysis of the nuLF power showed a significant main effect of sampling time ($F(1, 51) = 5.56$, $p < .01$, $\eta_p^2 = .25$) and a significant main effect of tDCS condition ($F(1, 53) = 6.37$, $p < .05$, $\eta_p^2 = .11$). This indicates a change in sympathetic activation over time as well as an overall difference between the tDCS conditions. The main effect cortisol response ($F < 1$) and the interaction of both between factors tDCS condition and cortisol response were not significant ($F < 1$). Similarly, the two-way interactions between sampling time and tDCS condition ($F < 1$) and between sampling time and cortisol response ($F(1, 51) = 1.55$, $p = .21$) are not significant, as well as the three-way interaction between all factors ($F < 1$).

nuHF analysis resulted in a significant main effect of sampling time ($F(1, 51) = 5.56$, $p < .01$, $\eta_p^2 = .25$) and a significant main effect of tDCS condition ($F(1, 53) = 6.75$, $p < .05$, $\eta_p^2 = .11$). This indicates a change in sympathetic activation over time as well as an overall difference between the tDCS conditions. The main effect cortisol response ($F < 1$) and the interaction of both between factors tDCS condition and cortisol response were not significant ($F < 1$). Similarly, the two-way interactions between sampling time and tDCS condition ($F < 1$) and between sampling time and cortisol response ($F(1, 51) = 1.55$, $p = .21$) are not significant, as well as the three-way interaction between all factors ($F < 1$).

With regards to the power-ratio of LF/HF bands, analysis revealed that only the main effect sampling time ($F(1, 51) = 4.09$, $p < .05$, $\eta_p^2 = .19$) and the main effect tDCS condition were significant ($F(1, 51) = 5.50$, $p < .05$, $\eta_p^2 = .01$), indicating that the balance between sympathetic and para sympathetic activity shifted over the course of the experiment and an overall difference between the tDCS conditions. The main effect cortisol response ($F < 1$) and the interaction of both between factors tDCS condition and cortisol response were not significant ($F < 1$). Similarly, the two-way interactions between sampling time and tDCS condition ($F < 1$) and between sampling time and cortisol response ($F(1, 51) = 2.18$, $p = .10$) are not significant, as well as the three-way interaction between all factors ($F < 1$). Since all HRV parameters are interlinked and show the same pattern of results, post-hoc simple contrasts against the measurement during the SECPT were only conducted for LF/HF. The analysis showed no significant difference in LF/HF between the measurement taken during the SECPT and any other sampling time. For details on HRV parameters see **Table 2.**

Correct Response RT. Only correct reactions that were longer than 200ms from cortisol-responders were considered for analysis. The RTs were submitted to a 2 (session: pre-tDCS vs. post-tDCS) x 2 (trial type: targets vs. distractors) x 2 (tDCS condition: anodal vs. sham) MANOVA. Out of the three main effects, the significant effects that emerged were the main effect of session ($F(1, 45) = 50.37$, $p < .001$, $\eta_p^2 = .53$) and trial type ($F(1, 45) = 10.37$, $p < .01$, $\eta_p^2 = .19$), while the main effect tDCS ($F(1, 45) = 0.277$, $p = .61$) showed no statistical significance. This indicated a significant improvement (i.e. a decrease) in correct RTs from session one to two and that overall participants responded faster to target trials. All two-way interactions showed no statistical significance: trial type x tDCS condition ($F(1, 45) = 0.03$, $p = .87$), session x tDCS condition ($F(1, 45) = 0.01$, $p = .91$) and trial type x session ($F(1, 45) = 0.34$, $p = .56$). There was no significant three-way interaction between the variables ($F(1, 45) = 0.05$, $p = .81$). Taken together this indicates that performance was only influenced (here: increased) by subsequent testing, which could be attributed to a positive stress effect but the performance was not modulated by the assigned tDCS condition. For a detailed overview see **Table 3.**

Accuracy. Wrong or missed responses were counted as errors. Only reactions that were longer than 200ms from cortisol responders were considered for analysis. The accuracy rates were submitted to a 2 (session: pre-tDCS vs. post-tDCS) x 2 (trial type: targets vs. distractors) x 2 (tDCS condition: anodal vs. sham)

MANOVA. Out of the three main effects, the significant effects that emerged were the main effect of session ($F(1, 45) = 20.10$, $p < .001$, $\eta_p^2 = .31$) and trial type ($F(1, 45) = 71.63$, $p < .001$, $\eta_p^2 = .61$), while the main effect tDCS ($F(1, 45) = 0.81$, $p = .37$) showed no statistical significance. That reflects that participants' responses to distractors were more accurate and that there was a general improvement in accuracy from session one to two. All two-way interactions showed no statistical significance: trial type x tDCS condition ($F(1, 45) = 0.21$, $p = .65$), session x tDCS condition ($F(1, 45) = 0.0002$, $p = .99$) and trial type x session ($F(1, 45) = 3.00$, $p = .09$). And there was no significant three-way interaction between the variables ($F(1, 45) = 0.23$, $p = .63$). Taken together this indicates that performance was only influenced (here: increased) by subsequent testing, which could be attributed to a positive stress effect but the performance was not modulated by the assigned tDCS condition. For a detailed overview see **Table 3.**

*Bayesian Analysis***.** To provide further support for the conclusion of an independence of the WM performance and tDCS, we opted for a Bayesian approach. To simplify interpretation of results we calculated pre-post differences values for each stimulation condition for four dependent variables (Δ(target accuracy), Δ(distractor accuracy), Δ(target RT), Δ(distractor RT)) and submitted them to a Bayesian independent sample t-test using JASP (Love et al., 2015). The null hypothesis states that there is no difference in performance change over time depending on the stimulation condition. We used a Cauchy prior distribution with r = .707 and Bayes factors for all tests provide anecdotal to moderate evidence for the null hypothesis (Jeffreys 1961; Wagenmakers et al., 2011). Specifically, a BF of 1-3 represents anecdotal and a BF of 3-10 reflect moderate evidence for the hypothesis. In detail, analysis reveals the following BFs for the null-hypothesis: BF_{01}Δ(target accuracy) = 3.33, BF_{01}Δ(distractor accuracy) = 2.84, BF_{01}Δ(target RT) = 3.41 and BF_{01}Δ(distractor RT) = 3.38. This means that the data is approximately 3 times more likely to occur under the null hypothesis than under the alternative hypothesis. Importantly, this effect was consistent across all measures. See **Figure A1** in the Appendix for details and assessment of robustness depending on prior width. Analysis of the physiological measures (i.e. nuLF, nuHF, sympatho-vagal balance and heart-rate) revealed anecdotal evidence for the null-hypothesis. In detail, for the cortisol concentrations BF_{01} range from 0.87 – 1.37 (BF_{10} range from 0.58 – 1.15), for nuLF BF_{01} range from 0.41 – 2.15 (BF_{10} range from 0.47 – 2.43), for nuHF BF_{01} range from 0.41 – 2.15 (BF_{10} range from 0.47 – 2.43) and for the sympatho-vagal balance BF_{01} range from 0.55 – 1.97 (BF_{10} range from 0.51 – 1.81). The strongest evidence for the null hypothesis comes from the heart-rate data; BF_{01} ranged from 2.42 – 3.40 (BF_{10} ranged from 0.29 – 0.41). Taken together this provides supporting, tentative evidence that there are no differences between tDCS groups in cortisol responders with regards physiological measures. See **Table A3** for a detailed listing of BFs.

Control Analyses. Control analysis were conducted in order to be sure that the groups were comparable and responded equally to the SECPT as well as the tDCS. The five aspects that were considered for the control analyses were the time that participants were able to keep their feet immersed in cold water, the subjective stress ratings, the subjective tDCS side-effects, the sex distribution across cortisol responders and non-responders, as well as the comparability of cortisol response between the stimulation groups. Overall, the time that participants were able to keep their feet in the water was not affected by either the tDCS condition ($F < 1$) or the cortisol response ($F < 1$), nor the interaction between the two ($F(1, 55) = 1.71$, $p = .20$). The four items of the subjective stress rating (i.e. rating unpleasantness, stressfulness, painfulness and difficulty to keep their feet immerged) were submitted to multivariate analysis with tDCS condition (anodal vs. sham) and cortisol response (responder vs. non-responder) being varied between subjects. Results showed that there was no significant effect of either tDCS condition ($F < 1$), cortisol response ($F(1, 52) = 1.70$, $p = .16$) or the interaction between the two ($F < 1$). Similarly, there was no significant effect of either tDCS condition ($F < 1$), cortisol response ($F < 1$) or the interaction between the two ($F(1, 51) = 1.62$, $p = .17$) on the subjective tDCS side-effects (i.e. itching, tingling, burning, headache and uneasiness). Furthermore, participants were asked to indicate the severity of the tDCS side-effects during the ramp-up phase, during the plateau-phase, and during the ramp-down phase. In line with previous results, neither the tDCS condition ($F < 1$), nor the cortisol response ($F < 1$) or the interaction between the two ($F(1, 52) = 1.45$, $p = .24$) significantly impacted the rating. Thus it can be assumed that ratings were not affected by the different experimental conditions. With regards to the sex-distribution depending on cortisol response, a chi^2-test was run. No association was found

between sex and cortisol response ($X^2(1) = .358$, $p = .55$)[2]. In order to confirm that the stimulation itself did not affect the cortisol response and to make sure the baseline level of cortisol was equal between groups; independent samples tests were carried out for each of the five cortisol-sampling points. Importantly, there was no different between the anodal and sham tDCS group at baseline level ($F(1, 57) = -1.39$, $p = .17$) or post stimulation before ($F(1, 57) = -1.75$, $p = .09$) or after ($F(1, 57) = -1.54$, $p = .13$) the second n-back session. This was also true when only cortisol responders were considered for analysis. For further details please refer to **Table A1** and **A2** in the appendix.

Discussion

The present paper explores the possibility of using non-invasive brain stimulation in order to reverse negative stress effects or further boost positive ones. Overall results are twofold: (1) the significant main effect of session and improvement in the lexical n-back task might be either interpreted as an increase in performance due to acute stress – as measured by an increase in cortisol – or due to practice (although even then one has to admit that stress did not prevent this learning effect), (2) among the cortisol responders anodal tDCS did not influence WM performance under the positive influences of stress (see **Table 3** and **Figure A1** in the appendix). In fact, we obtained evidence against the influence of tDCS.

The overall improvement in task performance after acute stress might be partially explained by the time of testing or practice. Firstly, with regards to the time of testing contrary to other studies that reported an impairment of WM functions (Oei, Everaerd, Elzinga, Van Well, & Bermond, 2006; Schoofs, Pabst, Brand, & Wolf, 2013; Schoofs et al., 2008) we tested our participants solely in the afternoon and not in the morning. This is important because, in the afternoon when baseline cortisol levels are low (Herman et al., 2005), an increase of cortisol after stress could elevate cortisol levels to a performance facilitating state. Put differently, one could say that in the afternoon participants were on the left side of the inverted U-shaped function relating cortisol release to performance, which means that moderate acute stress elevated cortisol levels enough to enhance performance due to an optimal level of activation (for details see De Kloet et al., 2005; Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Lupien et al., 2002). Secondly, there is evidence that n-back training improves task-specific performance (Au et al., 2015; Brem et al., 2018; Soveri, Antfolk, Karlsson, Salo, & Laine, 2017). The present data can somewhat disentangle the effect of practice and cortisol influence. While the group of cortisol-responders shows a significant pre-post improvement for RTs ($F(1, 45) = 50.37$, $p < .001$, $\eta_p^2 = .53$) and error rates ($F(1, 45) = 20.10$, $p < .001$, $\eta_p^2 = .31$), the cortisol non-responder showed only an improvement in RTs ($F(1, 10) = 14.38$, $p < .01$, $\eta_p^2 = .59$) but not in error rates ($F < 1$). Thus, we argue that stress, as measured by cortisol response, and not solely practice did help improve performance in the second n-back session.

Few previous studies have investigated the interaction of tDCS and stress effects. For example, Bogdanov & Schwabe (2016) also looked into the interaction of stress and tDCS effects on WM. They reported that overall, anodal online tDCS over the right DLPFC prevented the stress-induced WM impairment. Although the results of the present study are somewhat at odds with the aforementioned report, there are several important differences between the two studies to consider. Firstly, different WM tasks were used (Corsi block and digit span vs. lexical n-back task), which draw on different aspects of WM functioning (e.g. spatial and non-verbal vs. verbal WM). Secondly, the tDCS protocol differed significantly. While the present study used offline tDCS over the left DLPFC, Bogdanov & Schwabe (2016) used online tDCS of the right DLPFC. Additionally, Bogdanov & Schwabe (2016) used a larger 25cm² electrode and a somewhat lower current density (0.043 vs. 0.056 mA/cm²). Thus, their stimulation resulted in a weaker stimulation of larger area compared to the present study. Interestingly, a study by Ankri, Meiron, & Braw (2017) shows the opposite effect compared to Bogdanov & Schwabe (2016) and more similar results to the study at hand; they report that under stress, performance in a n-back task was enhanced. But surprisingly, participants that had received tDCS to the right DLPFC performed

2 It was considered to enter sex as an additional independent variable but since group sizes would become extremely unequal and small – severely limiting the interpretability of the results – the authors decided against it.

worse under stress compared to participants that received sham stimulation. Taken together, the results of these two studies and the present one do not paint a clear picture of how stress and tDCS effects interact. Future research should therefore aim to further explore and disentangle the relationship between stress and tDCS effects on cognitive functioning. One could also argue that the n-back task is not suited to show small interactions between stress and tDCS effects, and ideally a task should be used that produces reliable and replicable interactions with both interventions separately. Unfortunately, to the best of the authors knowledge no task or paradigm exists that procures robust effects that interact with both stress and tDCS interventions. To reliably observe interactions, future studies should implement tDCS and stress protocols that aim to maximize the effect; for example, using the SECPT over the CPT and using a refined stimulation protocol. Furthermore, researchers should aim to control for moderators and covariates (e.g. sex, time of testing, neurotransmitter balance, neurological or cardiovascular diseases, etc.).

There are several possible limitations to the present study. Firstly, we did not run a stress control group; the reasoning here was that anodal tDCS effects on WM performance are one of the best established tDCS effects in the literature. Still, one might argue – given the inconsistent pattern across the few studies examining the interaction between tDCS and stress – that a stress control group is needed to fully grasp the result pattern here. Secondly, the menstrual cycle has been shown to influence stress reactions (Hellhammer, Wüst, & Kudielka, 2009; Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999), but was not taken into account here, so this factor might have added noise to the result pattern. Thirdly, in the present paper tDCS was administered after the SECPT and before the second n-back session, so that the second n-back session is both influenced by the cortisol stress-response and tDCS after-effects. But one could argue that tDCS should have been administered during or before the SECPT to attenuate the stress response itself. Fourthly, in this analysis omission and commission errors were not separated. But false alarms and response omissions may be influenced differently by tDCS and stress (Mashal & Metzuyanim-Gorelick, 2019). Future studies should aim to disentangle the effects of neuromodulators and different types of errors and use this information to calculate the sensitivity index d'.

One issue that needs to be addressed is the replicability and robustness of tDCS effects. These inconsistent results with regards to tDCS modulation of cognition performance can in part be traced back to differences in methodology. There are several procedural variables that can be modified for a tDCS study; such as electrode positioning, electrode size, stimulation duration, and timing. These variations lead to less comparable studies and the difficulty in interpretation is oftentimes amplified by underpowered studies (Woods et al., 2016; Woods & Martin, 2016). Additionally, there is still no established standard in sham procedure. Successfully blinding participants with regards to the stimulation condition can be difficult if the experimental manipulation is associated with a somatosensory sensation (Ambrus, Paulus, & Antal, 2010; O'Connell et al., 2012; Roy, Sparing, Fink, & Hesse, 2015). Furthermore, there are also many inter-individual factors (e.g. pre-activation of the underlying area, sex, hormonal balance, neurotransmitter balance, brain morphology) influencing tDCS outcomes (Krause & Cohen Kadosh, 2014). Also, polarity specific tDCS effects are not fully understood yet; for example, there is a drastic lack of cathodal stimulation studies (Berryhill et al., 2014; Friehs & Frings, 2019a). Thus, it seems necessary for authors to follow best-practice guidelines as closely as possible and provide a detailed description and reasoning for their procedure.

To conclude, our findings show that anodal tDCS over the left DLPFC does not modulate WM functioning under acute stress influences – in detail, there is evidence against the influence of anodal offline tDCS on n-back task performance in cortisol responders. Although the present study has some limitations, these findings provide initial pieces of evidence that contribute to our understanding of the impact of stress on cognitive functions, specifically working memory, and how those stress-effects may or may not be modulated using non-invasive brain stimulation techniques.

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Appendix

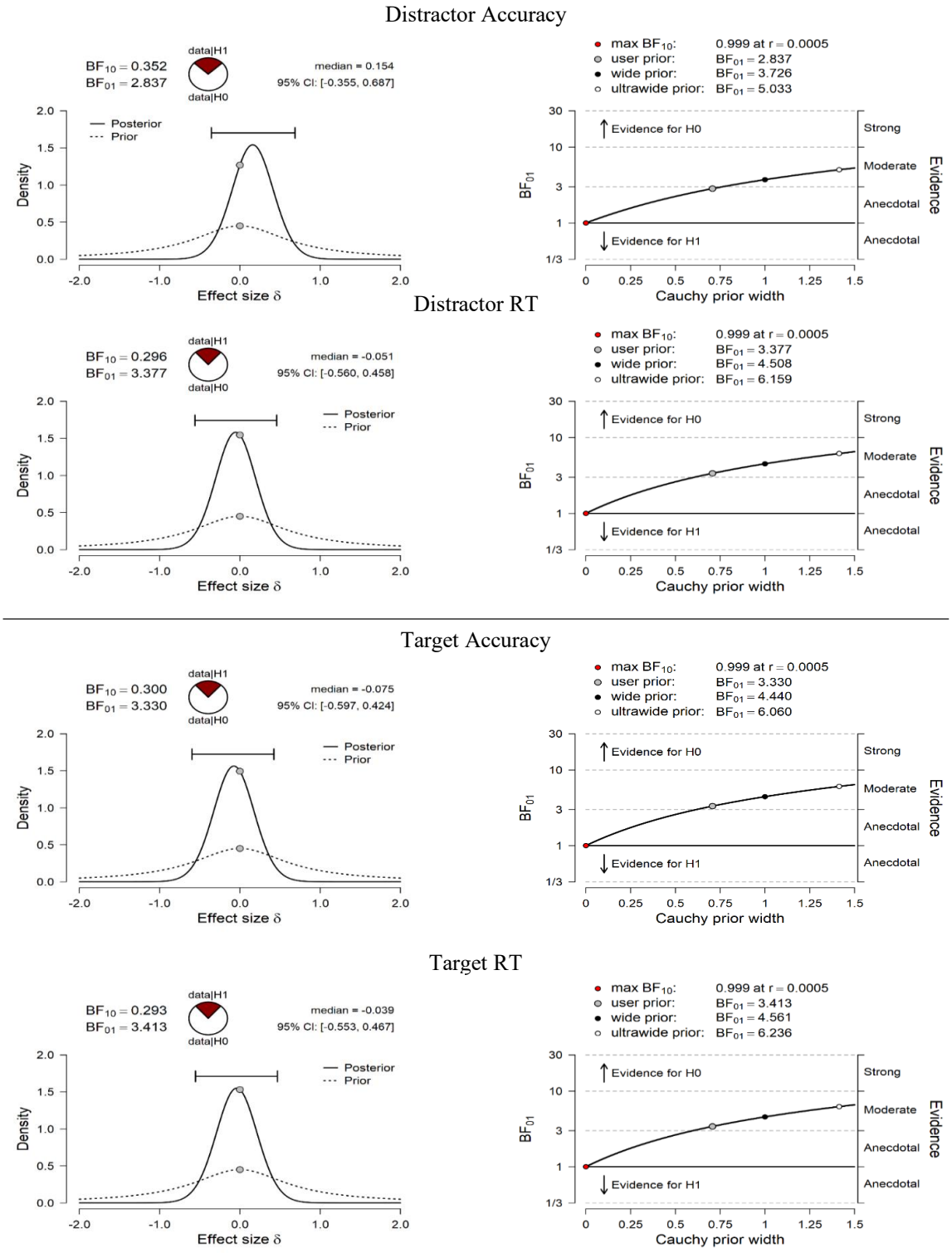

Figure A1. Details on Bayes factor calculation and robustness.

Table A1. independent samples test for the whole sample. tDCS groups were compared with regards to the measured cortisol concentration at different points in time.

	Test	Statistic	df	p
-35 min	Student	-1.394	57.000	0.169
	Welch	-1.399	54.816	0.167
	Mann-Whitney	330.000		0.114
0 min	Student	-1.562	57.000	0.124
	Welch	-1.574	48.811	0.122
	Mann-Whitney	355.000		0.228
+5 min	Student	-1.326	57.000	0.190
	Welch	-1.329	56.332	0.189
	Mann-Whitney	349.500		0.197
+20 min	Student	-1.748	57.000	0.086
	Welch	-1.767	41.813	0.085
	Mann-Whitney	374.500		0.363
+35 min	Student	-1.524	57.000	0.133
	Welch	-1.544	38.047	0.131
	Mann-Whitney	397.000		0.570

Table A2. independent samples test for the subgroup of cortisol responders. tDCS groups were compared with regards to the measured cortisol concentration at different points in time.

	Test	Statistic	df	p
-35 min	Student	-1.568	45.000	0.124
	Welch	-1.584	38.398	0.121
	Mann-Whitney	187.000		0.059
0 min	Student	-1.513	45.000	0.137
	Welch	-1.530	36.649	0.135
	Mann-Whitney	221.000		0.246
+5 min	Student	-1.301	45.000	0.200
	Welch	-1.306	44.326	0.198
	Mann-Whitney	214.500		0.194
+20 min	Student	-1.854	45.000	0.070
	Welch	-1.881	32.092	0.069
	Mann-Whitney	227.500		0.307
+35 min	Student	-1.571	45.000	0.123
	Welch	-1.597	29.861	0.121
	Mann-Whitney	242.000		0.476

Table A3. Bayesian independent samples t-test comparing both stimulation groups for the cortisol responders.

	BF_{01}	BF_{10}
cortisol concentration		
-35 min	1.283	0.779
0 min	1.372	0.729
+5 min	1.741	0.575
+20 min	0.873	1.145
+35 min	1.278	0.782
normalized low-frequency bands (nuLF)		
first n-back	2.151	0.465
during SECPT	1.611	0.621
during tDCS	0.664	1.507
second n-back	0.411	2.432
normalized high-frequency bands (nuHF)		
first n-back	2.151	0.465
during SECPT	1.611	0.621
during tDCS	0.664	1.507
second n-back	0.411	2.432
sympatho-vagal balance (LF/HF)		
first n-back	1.966	0.509
during SECPT	0.695	1.439
during tDCS	0.554	1.805
second n-back	1.047	0.955
heart rate in beats-per-minute (BPM)		
first n-back	2.416	0.414
during SECPT	3.404	0.294
during tDCS	3.283	0.305
second n-back	3.228	0.310